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EXPERIMENTS ON THE DEVELOPMENT OF THE LIMBS IN AMPHIBIA

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Although previous experiments by Barfurth,[1] Byrnes,[2] Kammerer,[3] Braus,[4] Lewis[5] and others have yielded many interesting facts regarding the development and regeneration of the limbs in Amphibia, the bounds of the limb rudiment at very early stages have not been determined exactly in any species, and it is not known how wounds of a definite size and character in the limb region affect the subsequent power to give rise to a normal appendage. In order to control properly certain transplantation experiments upon the fore limbs in *Amblystoma*, relating chiefly to questions of position and laterality, it was, therefore, found necessary to determine first the regenerative power of the tissues of the limb region after the removal of the limb rudiment in whole or in part before differentiation had begun. Accordingly the following experiments were made:

1. Simple extirpation of the body wall of the fore limb region at a period before the limb bud becomes visible on the surface, the size of the wound being varied.

2. Extirpation of a definite portion of the limb region.

3. Removal of the limb rudiment and subsequent covering of wound with ectoderm taken from some other region of the embryo.

4. Removal of the mesoderm alone from the limb region, afterward replacing the overlying ectoderm.

5. Removal of the ectoderm alone leaving the underlying mesoderm intact.

6. Transplantation of small masses of mesoderm, not yet visibly differentiated, from the limb region to pockets under the skin on the side of the embryo.

The fore limb appears at a much earlier stage than the hind limb in the Urodeles and much earlier than either extremity in the Anurans. The operations were consequently done upon embryos about the time

Table showing effect of removal of body wall of fore-limb region, the number of cases in each category being given

SIZE OF WOUND	WOUND NOT COVERED				WOUND COVERED			
	Not cleaned		Cleaned		Not cleaned		Cleaned	
	Regen.	Not regen.	Regen.	Not regen.	Regen.	Not regen.	Regen.	Not regen.
Not recorded	11	9			2	8		
3 somites	22	1	13	12			7	14
*3 somites							3	2
3½ somites			1	0			1	0
*3½ somites							0	1
3¾ somites	25	8	2	7			0	11
*3¾ somites							0	2
4 somites	16	2	2	3			0	3
*4 somites							0	2
4¼ somites	2	0						
Total	76**	20	18	22	2	8	11	35
Percentage	79	21	45	55	20	80	24	76

* In the groups so marked the ectoderm of the limb region was healed back in place; in the other groups in which the wound was covered ectoderm from other regions of the body was used.

** Includes five cases, constituting the fifth group of experiments, in which a large part of the mesoderm was purposely left in.

of appearance of the tail bud (fig. 1). In many cases, however, the embryos were slightly younger or slightly older than the one shown in the figure. The fore limb of *Amblystoma* develops as a thickening of the somatopleure which centers in the region of the fourth myotome and extends over into that of the third and fifth. In order to remove the tissues which form the appendage, a circular incision of about the diameter of these three segments was made with fine scissors, and the loosened piece including both ectoderm and mesoderm was then torn free and removed. The few mesoderm cells which are usually left sticking to the pronephros and around the edges of the wound were carefully removed in some cases and in others left in place. In some individuals the pronephros was left intact and in others it was removed. In nearly all cases the operation was done on one side, the other being left in its normal condition for comparison.

Experiments. 1. The results of the simple removal of the body wall of the limb region are given in the left half of the Table. While the figures in the single classes are often not large enough to give significant percentages, it is clear that the cleaning of the wound of all scattered mesoderm cells reduces very materially the proportion of cases in which regeneration occurs, i.e., from 79 to 45 per cent. It is also apparent that when the size of the wound exceeds in diameter the length of three somites the proportion of cases in which limbs develop is considerably less, whether the wound is cleaned or not. When it is carefully cleaned and exceeds three somites in diameter, the healing is slow and there is a very high mortality, which accounts for the small number of experiments with wounds of this size appearing in the tabulation.

The age of the embryos, within the limits of the stages experimented upon, has no influence upon the regenerative capacity. The presence of the pronephros, which on account of its close proximity might possibly

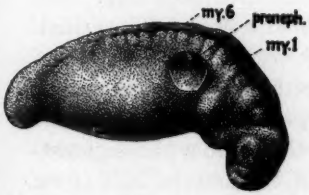

FIG. 1.

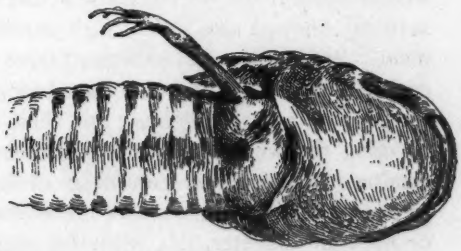

FIG. 2.

FIG. 1. EMBRYO OF AMBLYSTOMA PUNCTATUM SHOWING THE WOUND IMMEDIATELY AFTER OPERATION IN A TYPICAL EXPERIMENT. × 10.

FIG. 2. AMBLYSTOMA LARVA FROM WHICH THE LEFT FORE LIMB RUDIMENT WAS REMOVED, PRESERVED 87 DAYS AFTER OPERATION. × 5.

be thought to have some influence on the limb, seems not to affect its normal development, for a perfectly normal limb may be formed when the pronephros is removed. The removal of this organ facilitates, however, the cleaning away of the mesoderm cells and thus may affect indirectly the results of experiments, even though it exerts no formative stimulus.

The limbs which develop after removal of the rudiment are retarded in development to a varying degree. In no case has a limb been found subsequently to arise, when the negative condition has persisted till the twelfth day, and in only three cases when it has remained till the tenth. Consequently, if development has not begun by the end of the second week it is safe to assume that it will not take place at all. A large number of cases observed till the end of the third or the fourth week, and in some instances much longer, show this to be true (fig. 2).

No extensive examination of the material has been made by means of sections, but five cases which were cut show, that even though the free appendage is lacking, a shoulder girdle of somewhat reduced dimensions is developed on the operated side, just as Braus[6] has found in the case of *Bombinator*.

2. The experiments of the second group, in which limited portions of the limb rudiment were removed—dorsal, ventral, caudal, or rostral half, or the central portion—all resulted in the formation of perfect limbs whose development was but slightly retarded.

3. A comparison of the right and left halves of the Table shows at a glance that the covering of the wound with indifferent skin is a considerable hindrance to the development of the limb. The cases, cited on the first line of the table, in which the wounds were not cleaned happen to give, however, a considerably exaggerated idea of the effect of skin transplantation, since the control experiments in which the wound was left uncovered, made at the same time and with the same material, showed also a very high percentage of cases of non-development. Some unknown factor must have affected the results here. The cases in which the wound was cleaned are gathered, however, from experiments made at various times, and these show very clearly the effect of covering the wound. In the experiments with wounds of but three somites in diameter the percentage of development is reduced from 52 to 33, while in the cases where the wound is three and a half segments or more in diameter, i.e., when the region of the anterior half of the sixth segment is included, the percentage of development is reduced to zero. The determination of this latter point is of prime importance for the main purpose of this work, since it shows how much it is necessary to remove, when transplanting other buds to the limb region, in order to be sure that the appendage which develops is not simply regenerated from the host.

4. The experiments in which the ectoderm of the limb region was healed back in place after removal of the mesoderm are only ten in number and are, therefore, insufficient to give significant percentages. They nevertheless confirm the result of the previous series. Five experiments with wounds of three segments in diameter yielded three cases in which development occurred and two in which it did not occur. Five other experiments with wounds four segments in diameter were all negative. The mesoderm was carefully cleaned off at the time of the operation in all experiments of this set.

5. The five cases in which the ectoderm alone was removed and the mesoderm, at least in great part, left in place all resulted in the develop-

ment of a normal limb. Since these differ only in degree from those experiments of the first group in which the wound was not carefully cleaned, they have been included in the tabulation under that class.

6. To transplant the mesoderm alone, the ectoderm is first torn free from the underlying tissues after the usual circular incision is made. The mesoderm of the region is then cut out in a single piece as large as possible, and transferred with a blunt needle to a small pocket under the ectoderm of another embryo previously prepared. The mass of cells is then pushed into the pocket and left, a procedure that is not always easily carried out because of the extreme stickiness of these cells.

Nineteen experiments were made, in four of which the embryo died before yielding results. In five cases the transplanted tissue was entirely resorbed; in three others it remained a small nodule; in one a long appendage without digits resulted; and in six there developed a limb of approximately full size which, however, in most cases showed some irregularities, usually in the form of reduplications.

FIG. 3. AMBLYSTOMA LARVA WITH SUPERNUMERARY FORE LIMB, DEVELOPED FROM CELLS INOCULATED FROM THE MESODERM OF THE LIMB REGION OF AN EMBRYO IN THE STAGE SHOWN IN FIGURE 1. EXPERIMENT TR. MES. 1. PRESERVED 16 DAYS AFTER OPERATION. × 10.

The most perfect of these showed reduplication of only a single digit; another gave rise to a perfect limb with complete reduplication of fore-arm and manus. The one shown in figure 3 has the beginning of a supernumerary digit on the radial side of the hand. The other cases were more irregular. Sections show that in addition to the free appendage a small shoulder girdle is formed from the transplanted tissue. This is also the case when the entire limb rudiment is transplanted, as is shown both by Braus's[7] experiments upon *Bombinator* and my own upon *Amblystoma*.

Conclusions. The anterior limb of *Amblystoma*, according to the foregoing experiments, is already determined, at the time of appearance of the tail bud, in the mesoderm cells of that region of the body wall which lies close to the pronephros and ventral to the third, fourth, and fifth myotomes. The prospective significance of this group of cells as a whole thus is defined some time before differentiation becomes visible. Forming a narrow zone around this region there are cells which may vicariously give rise to a limb in case the rudiment proper is removed. These cells, responding to the stimulus of the wound, gradually move up toward the center, and covering the bare yolk, afterward in many

cases develop into a normal limb. Miss Byrnes[8] some years ago called attention to this phenomenon in the case of the hind limb rudiment of *Rana* embryos, though the results of the present study, which has aimed to determine exactly the limits of the power to regenerate, show that it is much more restricted than she supposed to be the case. Perhaps, however, there is in *Amblystoma* the same difference in regenerative capacity between fore and hind limb regions that Braus,[9] found to be the case in the Anurans. Covering the wound with indifferent ectoderm, or healing back the original ectoderm of the limb region, hinders this movement of peripheral cells toward the nodal point from which the new limb may arise, and definitely prevents development, provided that the extent of the wound includes the region of the anterior half of the sixth somite in addition to that of the third, fourth and fifth. In case the wound is but three segments in diameter, development may or may not be prevented but the proportion of negative cases is considerably larger than when the wound of the same size is left uncovered.

Carried out upon a different form and on much younger embryos than the experiments of Braus, the present experiments afford additional evidence that the mesoderm cells of the limb region, while forming a definitely differentiated system as a whole, are nevertheless totipotent within that system as far as the skeletal and muscular elements of the limb are concerned. They can give rise to a perfect limb when placed in an unusual environment, even when the original arrangement of the cells is disturbed, as it necessarily is when the mesoderm alone is transplanted; and a small part can develop into a whole. Other experiments, not described here, show, however, that at the time of operation differentiation has already begun to some extent, though it has evidently not become irreversible. The ectoderm merely serves as a covering to the limb and no indication of any specific stimulus from this layer could be detected. Other structures in the region such as the myotomes, as shown by Byrnes (1898) and Lewis (*op. cit.*) or the pronephros, as found in the present investigation, do not affect the development or regeneration of the limb bud in any way.

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[5] W. H. Lewis, *Anat. Record*, 4 (1910).
[6] H. Braus, *Morph. Jahrb.*, 39, 194 (1908).
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[9] H. Braus, *Morph. Jahrb.*, 35 (1906).

A MECHANISM OF PROTECTION AGAINST BACTERIAL INFECTION

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The means employed by the animal body to rid itself of bacteria have been conceived to be of two kinds: those of disintegration or lysis, and those of cellular inclusion or phagocytosis.

According to the former, the bacteria are acted upon by certain constituents of the blood serum—amboceptor and complement—which dissolve them; and according to the latter they are englobed by white blood corpuscles which digest them.

As a matter of fact, the first process has been inferred, rather than demonstrated. It is true that in shed blood the dissolution by lysis has been observed, but not in the living body. But even in shed blood or its serum constituents the solution occurs only with a part of the pathogenic bacteria, of which B. typhosus may be taken as an example. Such bacteria as pneumococcus, streptococcus, etc., are not subject directly to this form of dissolution. Phagocytosis, on the other hand, is a more general phenomenon and applies to a wide variety of bacteria.

It has long been known that when bacteria are introduced into and later disappear from the blood, they are not eliminated by the organs of excretion, but are destroyed in the organs themselves. The problem at issue relates to the manner of the destruction.

The question should be considered with reference to two states of the animal body, namely, the unprotected or normal, and the protected or immune state.

Taking certain forms of pathogenic or disease producing bacteria, a study was made as to the manner of their disappearance in protected rabbits. The pneumococcus and typhoid bacillus may serve as examples. Protection was secured by the employment of immune sera. In the case of the pneumococci, the type of pneumococcus and immune serum must coincide. In the experiments a type I pneumococcus and corresponding serum were employed.

Protection against pneumococcus. It has been shown that an active pneumococcus serum protects against a certain maximum quantity of pneumococcus culture, but that multiples of the serum do not protect equally against multiples of the culture. An effective culture of pneumococcus causes on inoculation fatal septicemia in the rabbit, followed

by death in 24 to 48 hours or less. When an immune serum is employed, life may be saved or the surviving period merely prolonged.

The immediate effect of a serum injection is to cause the removal of the pneumococci from the circulating blood. This effect is produced in an incredibly short period of time—in a few minutes indeed. But the permanency of the removal depends in part on the quantity (or dose) of antiserum injected. Small doses of serum are more effective than large doses, and the former may be successful in saving life, while the latter are not.

The mechanism of the removal is as follows: when an immune serum is introduced into the blood of a rabbit suffering from pneumococcus septicemia, an almost immediate agglutination of the bacteria takes place. The larger the doses of the serum, within limits, the larger the size of the bacterial clumps that are found. The clumps are removed from the blood almost immediately by the organs—the spleen, liver, and bone marrow. What happens next is determined by the size of the clumps. If they are large, they cannot be ingested by phagocytes; hence they soon begin to multiply, and the bacteria reinvade the blood; if small, they are taken up by phagocytes and are digested. The animal succumbs on the one and survives on the other hand. Hence small doses of the serum causing smaller clumps may be more effective than large doses giving larger ones. No extra-phagocytic dissolution of the pneumococci seems to occur.

Protection against the typhoid bacillus. A similar mechanism operates in the rabbit inoculated with cultures of the typhoid bacillus. The typhoid bacilli, notwithstanding the fact that they are subject to serum-lysis, are taken out of the blood by the organs after clumping, and the clumps are ingested by phagocytes which digest them.

Pathogenic and non-pathogenic bacteria. Certain cultures of disease-producing bacteria are not, others are pathogenic for animals. The influenza bacillus appears in these two distinct varieties. When cultures by the non-pathogenic variety are injected into the circulation of rabbits, they are clumped and removed by the organs at once; when cultures of the pathogenic variety are inoculated, they are neither clumped nor removed. Hence a pathogenic effect may depend upon agglutinability of the bacteria—by the blood of normal or of immunized animals.

In other words, bacteria circulating in the blood are quickly removed when they are agglutinated or clumped, and the clumps deposited within the organs are taken up by phagocytes and digested. They appear not to be destroyed by solution or lysis through the operation of serum constitutents of the blood.

ON THE LIFE-HISTORY OF *GIARDIA*

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This paper is based upon the examination of 220 mammals, gray and white culture mice (98), culture rats (42), and wild *Peromyscus maniculatus gambeli* (Baird) (59), *Microtus californicus californicus* (Peale) (6), and coyotes [Canis ochropus ochropus (Peale)] (2) trapped near Berkeley. About 13% of the total were infected, 22 of the mice, and 4 of the *Peromyscus*, with *Giardia muris* and three of the *Microtus* with *G. microti* sp. nov. Several hundred slides prepared by the Schaudinn-iron haematoxylin wet method were examined and stages in binary and multiple fission and in encystment obtained in quantity.

In the last edition of Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen* Jollos (1913) denies the existence of multiplicative phases in *Lamblia* in the free stage in the intestine of the host. Rodenwaldt (1911) had also held this view in Prowazek's *Handbuch der pathogenen Protozoen* and Alexeieff (1914) and Prowazek and Werner (1914) as the result of recent investigations have confirmed it. Castellani and Chalmers in the last edition (1913) of their *Manual of Tropical Medicine* accept Noc's (1908) conclusion, seemingly made without study of comparative material, that there is only one species concerned in the intestinal infections alike of mice, rats, cats, and man, and make the inference that such vermin become sources of infection to man by the contamination of his food and water with their cyst-bearing faeces.

It comes thus to be of interest to human and comparative medicine to learn whether or not these intestinal flagellates which produce a chronic enteritis in young mice and are under suspicion of similar activity in man, may multiply in the free state in the intestine, or whether infection is limited to the number of ingested spores, and especially to learn whether there are separate species restricted to given host species or groups of species of rodents, and another species found in man. If the latter be the case, the probability of these mammals being sources of infection is reduced considerably, unless it be that the parasites transform from one 'species' to another with change of host. The biological problems of host specificity, and transformation by environment are thus involved in this question.

It is the purpose of this paper to demonstrate the occurrence of binary and multiple fission in *Giardia* (=*Lamblia*), in both the free (hitherto denied) and encysted stage, to describe mitosis in these minute organ-

isms, to offer confirmatory evidence for the presence of several species instead of one only in the genus, to add to the skepticism regarding autogamy and the existence of an *Octomitus* stage in the life-history, advanced by Hartmann (1911), and to offer some inconclusive evidence of maturation in the so-called conjugation cysts. The paper will be published in full in the University of California Publications in Zoology.

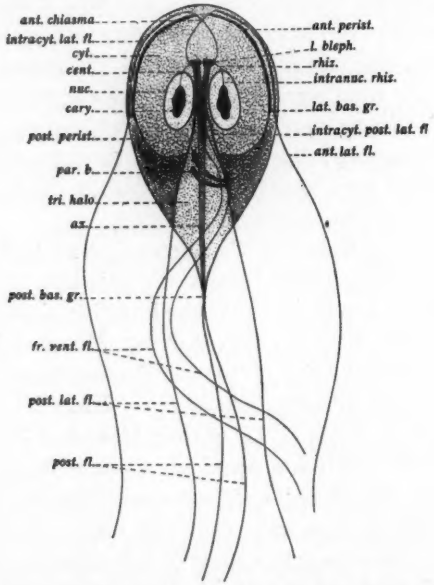

Fig. 1. Ventral view of *Giardia microti* sp. nov. in early prophase of mitosis, somewhat diagrammatic. × 3400. Abbreviations: *ant. chiasma*, anterior chiasma; *ant. lat. fl.*, anterior lateral flagellum; *ant. perist.*, anterior peristome; *ax.*, axostyle; *cary.*, karyosome; *cent.*, centrosome; *cyt.*, cytostome; *fr. vent. fl.*, free ventral flagellum; *intracyt. lat. fl.*, intracytoplasmic part of the antero-lateral flagellum; *intracyt. post. lat. fl.*, intra-cytoplasmic part of the postero-lateral flagellum; *intranuc. rhiz.*, intranuclear rhizoplast; *lat. bas. gr.*, lateral basal granule of antero-lateral flagellum; *l. bleph.*, left blepharoplast; *nuc.*, nucleus; *par. b.*, parabasal body; *post. bas. gr.*, posterior basal granule of posterior flagella; *post. fl.*, posterior flagella; *post. lat. fl.*, posterolateral flagellum; *post. perist.*, posterior peristome; *rhiz.*, rhizoplast joining nucleus to blepharoplast; *tri. halo:* triangular halo about posterior part of the axostyle.

Giardia microti sp. nov. (fig. 1) in common with the other species, is a diplozoic flagellate, a binucleate somatella with eight flagella in four pairs, antero-lateral, postero-lateral, free ventral, and posterior, the last emerging from the posterior tip of the axially located axostyle, the ventrals from the ventral surface, and the others from the lateral mar-

gins. The flagella form a part of an integrated neuromotor system of fibrils which unite the two component cells by a single chromatic system staining more or less deeply with iron haematoxylin. This system consists of a rhizoplast from the centrally located karyosome of each nucleus which passes through the centrosome on the nuclear membrane to the blepharoplast on its side at the head of the axostyle.

From the two blepharoplasts the single axostyle (not two as heretofore reported) passes posteriorly to emerge as the posterior pair of flagella. From the blepharoplast or near it, also arise the other flagella. The antero-lateral passes forward within the cytoplasm, crosses its mate of the opposite side uniting at the crossing in a node, the anterior chiasma, and thence curves posteriorly along the margin of the cytostome to emerge from the lateral basal granule as a free antero-lateral flagellum. The postero-lateral passes obliquely posteriorly within the cytoplasm to emerge near the margin, and the free ventrals emerge at once on the ventral surface. The blepharoplasts of the two sides of the axostyle are united across its head by a transverse bridge commissure. The antero-lateral flagella run in close conjunction with the thickened chromatic anterior peristome and the postero-laterals are in contact with the posterior arc of the peristome. This thickened chromatic margin lies in the rim of the deeply cupped ventral cytostome, the organ of attachment of the parasite.

Dorsal to the axostyle is a pair of chromatic bodies, the parabasals which contain stainable material whose volume fluctuates with the conditions of metabolism and which may function as reservoirs of cytoplasmic chromidial material. They appear to have a characteristic shape in each species. In *G. muris* they are stout ellipsoidal, and nearly fused. In *G. microti* (fig. 1) they are slender, curved, tapering, parallel structures. In *G. muris* each is connected with the axostyle near the blepharoplast by a slender chromatic fibre.

These organisms multiply in both the free and the encysted stages, by simple binary fission, and by multiple fission leading to the formation of a 16-nucleate, 8-zooid plasmodium or somatella, or a 16-nucleate, potentially 8-zooid cyst. Binary fission occurs by a well defined mitosis followed by a delayed plasmotomy.

Mitosis in *Giardia* is much like that described by Kofoid and Swezy (1915) for *Trichomonas*. It begins in the blepharoplast whose division is followed by that of the axostyle which splits distally to form two on the anterior ends of which the daughter blepharoplasts are attached. The centrosome at the upper pole of the nucleus divides and the daughters assume polar positions on the membrane of the elongating nucleus,

spinning out a temporary paradesmose between them upon the outside of the persistent nuclear membrane. The nucleus, from which an intranuclear chromidial cloud had emerged, with subsequent increase of cytoplasmic chromidia, exhibits an enlargement of the karyosome, its breaking up into eight chromatic granules probably representing four precociously split chromosomes which form in parallel rows and merge into a split skein from which four chromosomes emerge in the equatorial plate at the metaphase. These constrict, the daughter chromosomes migrate toward the poles of the intranuclear spindle, and the nuclear membrane constricts the parent into two daughter nuclei.

Plasmotomy is long delayed. The separation is longitudinal, the two daughter nuclei of each side forming the nuclei of the daughter zooids, which hang together assuming a great variety of positions before the protoplasmic bridge finally parts.

Multiple fission is of three types. The first is in free individuals in which three mitoses follow in succession before plasmotomy occurs and each zooid during the process acquires its full complement of organelles before the next division ensues. The result is a 16-nucleate, free-moving plasmodium composed of 8 fully equipped zooids which they separate singly. These small individuals are not *Octomitus*-like and are not to be confused with *Hexamitus muris* Grassi (=*Octomitus*), which species occurs in the same host with *G. muris*. *Giardia*, according to our evidence, does not pass through an *Octomitus* stage as Hartmann (1911) proposed.

Multiple fission in free forms occurs also by nuclear division with which the extranuclear organelles do not keep pace in multiplication, so that free individuals with the normal single equipment of these of extranuclear organelles are crowded with 16 nuclei in a bilateral or ultimately an irregular arrangement. The later history of such individuals has not been traced.

Single encysted individuals may also undergo multiple fission. The extranuclear organelles tend to disappear, the axostyle and posterior peristome lingering longer than the other chromatic parts as the nuclei go through three succeeding divisions producing a 16-nucleate mass within the cyst. Such cysts seem not to undergo further development in the rectum of the host in which they are found.

We find no evidence that the so-called conjugation cysts contain two sister individuals, and that therefore autogamy occurs. Pairs of individuals in end-to-end back-to-back position as in cysts are found free without evidence of their origin by fission. This condition may precede encystment. The two individuals which may simulate maturation

divisions in so far that the first division of each nucleus gives rise to two nuclei, one with abundant chromatin, and one (first polar nucleus?) with little chromatin. The second division occurs in both of these daughter nuclei. The smaller nucleus divides forming two still smaller nuclei and the larger one forms one nucleus rich in chromatin (the gamete nucleus?) and one with little chromatin (the second polar nucleus?). However, no evidence of reduction in number of chromosomes is demonstrable. It is obvious that, since these divisions occurred in both nuclei of the somatella, we might have a binucleate gamete, and should the two 'conjugants' in the cyst unite, a binucleate zygote, a condition highly improbable. The alternative explanation of this simulation of maturation is that we have here some form of degenerative multiple fission but the evidence of this is not conclusive.

We find evidence of groups of structural characteristics which are diagnostic of the several species in the genus. The same organelles with the same fundamental relations of position occur in all of the species at comparable stages. The specific differences consist in modifications in shape, size, proportions, relative size and shape of the cytostome and its relation to the margin of the body, in the size, shape, and position of the parabasal bodies, in general stainability, and in relations to hosts. It is possible thus to characterize *G. agilis* Alexeieff from the tadpole, *G. sanguinis* (Gonder) from *Elanus*, a South African falcon, *G. duodenalis* (Davaine) from the European rabbit, *G. muris* (Grassi), from *Mus*, *Epimys*, *Arvicola* (?) and *Peromyscus*, *G. microti*, sp. nov. from *Microtus*, and the species widely known as *Lamblia intestinalis* (Lambl) from man. Conclusions as to the identity of *Giardia* (= *Lamblia*) of man and rodents now generally accepted are of doubtful validity and require critical verification.

The generic name *Lamblia* Blanchard 1888 should give way to *Giardia* Kunstler 1882 on ground of priority since species of the two are morphologically so alike that no generic distinction can be made between them.

Our work has shown that *Giardia muris* and *G. microti* produce a readily recognizable enteritis in mice, and that both binary and multiple fission take place in the free non-encysted stage. It may be found abundantly in material well smeared out from the intestinal epithelium. Multiple fission results in a 16-nucleate, 8-zooid plasmodium which later undergoes disintegrative plasmotomy. There is no *Octomitus* stage in the life-history of *Giardia*. There is typically one axostyle in the trophozoite. Prophase stages in which this has divided to form two axostyles are often seen. The so-called 'Rätzelkörper' are homo-

logues of the parabasals of the trichomonads. The extranuclear organelles are united with the karyosome, centrosome, and blepharoplast in an integrated neuromotor apparatus. Mitosis is intranuclear, with precocious splitting of the four chromosomes which subsequently fuse in four in the equatorial plate. Free pairs of individuals are found united in back-to-back position as in the so-called conjugation cysts. Nuclei in these cysts undergo two divisions simulating reduction divisions in which, however, chromosomes reduction has not been demonstrated. No evidence in support of autogamy and no proof of sexual reproduction has been discovered.

Morphological characters separate six species in *Giardia*. The parasite in mice appears to be distinct from that in man. The generic name *Giardia* Kunstler should supersede *Lamblia* Blanchard on grounds of priority.

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THE INORGANIC CONSTITUENTS OF ALCYONARIA

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UNITED STATES GEOLOGICAL SURVEY, WASHINGTON

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The zoantharia, madreporaria, or stony corals have been repeatedly analyzed, and with generally concordant results. Thirty analyses, made in the course of the present investigation, of which this paper is a preliminary notice,[1] have confirmed the older data. These corals consist mainly of calcium carbonate, with one or two per cent of minor impurities, and a little organic matter. The same is true of the coralline hydrozoa, of which six analyses, representing the genera *Millepora* and *Distichopora*, have also been made. The alcyonaria, however, which include the red corals, the gorgonias, and other fan-like or branching forms, are quite different; and they are generally characterized by the presence in them of magnesium carbonate, and often of calcium phos-

phate also. Many of them, moreover, are rich in organic matter. To these statements there is one known exception. The blue coral, *Heliopora cerulea*, is an isolated organism, closely resembling the true corals, and like them in its chemical composition.

In the course of our research upon the inorganic constitutents of marine invertebrates, 22 analyses of alcyonarians have been made, representing the following species:

1. *Heliopora cerulea*, Pallas. Blue coral. Southern Philippines.
2. *Tubipora purpurea*, Lamarck. Singapore, Straits Settlements. Latitude 1° 20′ N.
3. *Corallium elatior*, Ridley. A red coral. Murotsu, Tosa, Japan. Latitude about 33° N.
4. *Primnoa reseda*, Verrill. East of Nova Scotia. Latitude 44° 16′ N.
5. *Lepidisis carophyllia*, Verrill. Off Nantucket shoals. Latitude 38° 53′ N. Depth of water 3,168 metres. Temperature 3°. 3′. C.
6. *Pennatula aculeata*, Dana. St. Peter's Bank. Latitude 44° 47′ N. Temperature 4°. 5 C.
7. *Paramuricea borealis*, Verrill. Southwest edge of the Grand Bank.
8. *Paragorgia arborea*, Milne-Edwards and Haime. Le Have Ridge, off Nova Scotia.
9. *Alcyonium carneum*, L. Agassiz. Southwest of Stellwagen Bank, off Race Point Light, Cape Cod, Massachusetts. Temperature 3°. 9 C.
10. *Gorgonia suffruticosa*, Dana. Fiji Islands.
11. *Gorgonia acerosa*, Pallas. Nassau, Bahamas. Latitude 25°, 5′ 6″ N.
12. *Gorgonia acerosa*, Caesar's Creek, Southern Florida. Latitude about 23° 30′ N.
13. *Muricea humilis*, Milne-Edwards. Parahyba do Norte, Brazil.
14. *Muricea echinata*, Valenciennes. Cape San Lucas, Lower California. Latitude 22° 52′ N.
15. *Plexaurella grandiflora*, Verrill. Mar Grande, Bahia, Brazil.
16. *Ctenocella pectinata*, Valenciennes. Torres Straits, Australia. Latitude about 10° S.
17. *Xiphogorgia anceps*, Pallas, Caesar's Creek, Florida.
18. *Rhipidogorgia flabellum*, Linné, Bermuda. Latitude about 32° N.
19. *Rhipidogorgia flabellum*, Andros Island, Bahamas. Latitude about 25° N.
20. *Leptogorgia pulchra*, Verrill. La Paz, Gulf of California. Latitude 24° 16′ N.
21. *Leptogorgia rigida*, Verrill. Cape San Lucas, Lower California.
22. *Phyllogorgia quercifolia*, Dana. Fernando de Noronha, Brazil. Latitude 3° 50′ S.

The complete analyses of these alcyonarians, which will be published in our final report, show very variable proportions of organic matter.

In Nos. 1, 2, 3, and 5, very little was found; while the others contained quantities ranging from 13 to 61%. Rejecting this variable constituent, and recalculating the inorganic portion to 100%, the reduced or rational analyses assume the following form:

	1	2	3	4	5	6	7	8
SiO_2	0.15	1.40	0.00	0.13	0.11	1.70	0.44	0.15
$(Al,Fe)_2O_3$	0.07	0.57	0.15	0.88	0.05	1.01	0.30	0.03
$MgCO_3$	0.35	12.23	11.56	6.18	6.92	7.71	8.03	9.05
$CaCO_3$	98.93	84.61	86.57	90.39	92.24	85.62	85.11	88.04
$CaSO_4$	0.50	1.19	1.32	1.59	0.68	0.84	4.69	2.17
$Ca_3P_2O_8$	trace	trace	0.40	0.83	trace	3.12	1.43	0.56
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	9	10	11	12	13	14	15
SiO_2	18.05	0.55	0.22	0.04	0.56	0.11	0.45
$(Al,Fe)_2O_3$	5.80	0.28	0.22	0.24	0.07	0.06	0.15
$MgCO_3$	9.21	13.43	12.52	13.29	12.64	12.28	13.79
$CaCO_3$	52.23	79.84	81.45	79.48	84.47	83.79	85.61
$CaSO_4$	1.36	5.43	1.95	4.08	1.73	2.93	trace
$Ca_3P_2O_8$	13.35	0.47	3.64	2.87	0.59	0.83	trace
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	16	17	18	19	20	21	22
SiO_2	0.21	0.14	0.21	0.24	0.09	0.28	0.34
$(Al,Fe)_2O_3$	0.13	0.07	0.28	0.07	0.03	0.21	0.26
$MgCO_3$	15.65	13.04	12.64	13.19	13.71	14.13	15.73
$CaCO_3$	81.44	80.96	63.38	80.75	74.99	75.36	72.99
$CaSO_4$	1.69	3.83	2.40	2.95	2.91	2.07	2.11
$Ca_3P_2O_8$	0.88	1.96	1.09	2.80	8.27	7.95	8.57
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Analysis No. 1, of *Heliopora*, might be that of an ordinary coral, being almost non-magnesian and therefore different from all the others. The material of No. 9, *Alcyonium*, was obviously impure, with much admixture of sand and mud. That the species is remarkably rich in phosphate, however, seems to be clear; but it needs further investigation upon a better sample. In two of the *Gorgonias* the black, wiry axis was separately examined, although there was not enough of it for satisfactory analysis. In *G. suffruticosa* the axis lost on ignition 94.39%, and in *G. acerosa* 96.35%, mostly organic matter. The inorganic residues were small in amount, but partial analysis showed that they differed in composition from the more abundant calcareous envelopes. In some genera the axes are mainly calcareous; but in others the organic centers are very large, and the species are difficult to deal with analytically. Further investigation upon the differences in composition between cortex and axis is needed, but on biological rather than geological grounds. As the purpose of this investigation is to determine what each group

of organisms contributes to the formation of marine limestones, an extended study of their organic matter would be hardly relevant.

If, now, we arrange the alcyonaria in the order of ascending magnesium carbonate, a remarkable relation appears connecting composition with the temperature of the habitat. *Heliopora*, being anomalous, is not included in the table. *Alycyonium* is also excluded, on account of its impurities. The percentages of $MgCO_3$ and $Ca_3P_2O_8$ are both given.

Magnesium carbonate and calcium phosphate in alcyonaria.

SPECIES	LOCALITY	LATITUDE	$Ca_3P_2O_8$	$MgCO_3$
Primnoa reseda	Nova Scotia	42° 16′ N.	0.83	6.18
Lepidisis caryophyllia	Off Nantucket	48° 53′ N.	trace	6.92
Pennatula aculeata	Nova Scotia	44° 47′ N.	3.12	7.71
Paramuricea borealis	Grand Banks		1.43	8.03
Paragorgia arborea	Nova Scotia		0.56	9.05
Corallium elatior	Japan	33° N.	0.40	11.56
Tubipora purpurea	Singapore	1° 20′ N.	trace	12.23
Muricea echinata	C. San Lucas	22° 52′ N.	0.83	12.28
Gorgonia acerosa	Bahamas	25° 5′ N.	3.64	12.52
Muricea humilis	Brazil	7°–8° S.	0.59	12.64
Rhipidogorgia flabellum	Bermuda	32° N.	1.09	12.64
Xiphogorgia anceps	Florida	22° 30′ N.	1.96	13.04
Rhipidogorgia flabellum	Bahamaas	25° N.	2.80	13.19
Gorgonia acerosa	Florida	22° 30′ N.	2.87	13.29
Gorgonia suffruticosa	Fiji		0.47	13.43
Leptogorgia pulchra	L. California	24° 16′ N.	8.27	13.71
Plexaurella grandiflora	Brazil		trace	13.79
Leptogorgia rigida	C. San Lucas	22° 52′ N.	7.95	14.13
Ctenocella pectinata	Torres Straits	10° S.	0.88	15.65
Phyllogorgia quercifolia	Brazil	3° 50′ S.	8.57	15.73

Although records of temperature and depth of water are available for only a few of these alcyonaria, the suggested relation is clear. The organisms from cold, northern waters, or from very deep waters, are low in magnesia, while those from warm regions are much higher. The same relation appears in our analyses of echinoderms, and is unmistakable; even though it is as yet unexplained. It is not rigorously exact; but some apparent irregularities are due to the disturbing effect of impurities, such as sand or mud, which appear in the analyses as silica and sesquioxides. If these were rejected the percentage of magnesia would be raised. Variations are also to be expected because of cold or warm currents, and differing depths of water. Very deep water, even under the equator, is always cold; while shallow bays, even quite far north, may be relatively warm. Possibly also, the alcyonaria may form several distinct series, not perfectly comparable as regards chemical

composition. *Corallium* and *Tubipora*, for example, are compact forms, with little organic matter; and they are lower in magnesia than the genera with horny, organic axes, such as appear at the end of the table. It is also noteworthy that the highest proportions of calcium phosphate are commonly found associated with high values for magnesia.

[1] Published by permission of the Director of the U. S. Geological Survey.

AN EXPERIMENTAL ANALYSIS OF THE ORIGIN AND RELATIONSHIP OF BLOOD CORPUSCLES AND THE LINING CELLS OF VESSELS

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Studies on the origin and development of the cellular elements of the blood and the so-called endothelial cells which line the blood vessels in the normal embryo are peculiarly difficult on account of the important rôle that wandering mesenchyme cells play in these processes. The problem is also further confused by the perplexing mixture of cells of different origin brought about by the early established circulation of the body fluids. The development of no other embryonic tissue is so disturbed by mechanical and physical conditions.

A study of living fish embryos with the high power microscope has made it possible to observe the behavior of the wandering cells and to follow them in their development. The disadvantages due to the intermixture of cells in the blood current have been overcome by the investigation of embryos in which a circulation of the blood is prevented from taking place.

When the eggs of the fish, Fundulus heteroclitus, are treated during early developmental stages with weak solutions of alcohol, the resulting embryos in many cases never establish a blood circulation. In other respects these embryos may be very nearly normal and the development and differentiation of their tissues and organs often proceed in the usual manner, though at a somewhat slower rate. The heart and chief vessels are formed and the blood cells arise and develop in a vigorous fashion. The heart pulsates rhythmically but is unable to propel the body fluid since its venous end does not connect with the yolk vessels. And in many cases its lumen is partially or completely obliterated by periblastic material and nuclei which seem to be sucked into the heart cavity from the surface of the yolk.

In these embryos without a circulation of the blood, one is enabled

to study the complete development of the different types of blood corpuscles in the particular regions in which they originate. There is no contamination of the products of a given region through the introduction of foreign cells normally carried in the blood stream.

The actual power to form blood cells possessed by the different organs and tissues may be determined in the experimental embryos having no blood circulation. And the true haematopoetic function is thus clearly contrasted with the ordinary reproduction or multiplication of blood cells which might take place within the tissue spaces of such organs in the normal embryo.

The debated question regarding the production of blood cells from those cells lining the blood vessel wall may be convincingly answered, at least for the species here studied.

The results and conclusions derived from these experiments may be summarized as follows:

1. The fish embryo is capable of living and developing in an almost normal fashion without a circulation of its blood; this fact was first recorded by J. Loeb in 1893, yet this is the initial study of blood and vessel formation in such embryos. Red blood cells may be seen to arise and differentiate in these living embryos in two definite localities; the one within the posterior body region, and the other the blood islands on the yolk.

The blood cells remain confined to their places of origin, yet they attain a typical red color and may persist in an apparently functional condition on the yolk-sac for as long as sixteen to twenty days. The normal embryo becomes free swimming at from twelve to fifteen days, but individuals without a circulation never hatch although they may often live for more than thirty days.

All recent investigators have claimed that there are no blood islands present on the bony fish yolk-sac. Yet the presence of such islands is readily demonstrated in living Fundulus embryos, in the normal as well as in those with no circulation.

2. The plasma or fluid in the embryos which fail to develop a circulation begins to collect at an early time in the body cavities. The pericardium becomes hugely distended with fluid, as well as the lateral coelomic spaces and the Kupffer's vesicle at the posterior end of the embryo. The great distension of the pericardium due to this fluid accumulation pushes the head end of the embryo unusually far away from the surface of the yolk. The heart is thus often stretched into a long straight tube or string leading from the ventral surface of the head through the great pericardial cavity to the anterior yolk surface.

No blood vessels, or very few if any, form on the extreme anterior portion of the yolk-sac, so that the venous end of the heart is never connected with veins. And the heart does not draw fluid into its cavity to be pumped away through the aorta. When the heart cavity does contain fluid it is unable to escape and small floating particles may often be observed rising and falling with the feeble pulsations.

3. The hearts in embryos without a circulation are lined with a definite endocardium, but the myocardium or muscle wall is poorly developed, sometimes consisting of only a single cell layer. Pigmented cells are not present in the wall of the normal heart, but in the experimental hearts these large chromatophores are invariably found. The cavity in many of the hearts is almost if not entirely obliterated by the presence of periblastic material and huge amorphous periblast nuclei.

The conus end of such hearts leads directly to a more or less closed ventral aorta. Portions of the aortic arches are seen as open spaces, and the dorsal aortae are almost invariably seen as typical spaces lined by characteristic embryonic endothelium.

A point of much importance is the fact, that *neither these hearts with their endothelial linings nor any portion of the aortae at any stage of development have ever been seen to contain any form of red blood corpuscle.* Cells of this type are completely absent from the anterior regions of the embryo.

4. Pigment cells normally occur on the Fundulus yolk-sac and arrange themselves along the vascular net so as to map out the yolk-sac vessels in a striking manner. Loeb has thought that this arrangement along the vessel walls was possibly due to the presence of oxygen carried by the corpuscles within the vessels. In the embryos without a yolk-sac circulation the pigment cells arise but rarely become fully expanded so that the usual long branched processes are represented only by short projections; the chromatophore consequently seems much smaller than usual.

The unexpanded pigment cells, however, wander over the yolk-sac and collect in numbers around the plasma filled spaces. The yolk surface of the pericardium and the periphery of the Kupffer's vesicle are often almost covered with pigment. The hearts are during early stages full of plasma and the pigment cells form a sheath around them, while such cells are never present on the normal hearts during the embryonic period.

These facts would seem to indicate that the plasma rather than the blood corpuscles contains the substance which attracts the chromatophores and initiates their arrangement along the normal vascular net of the yolk-sac.

5. A definite mass of cells characteristic of the fish embryo is located in the posterior half of the body between the notochord and the gut and extends well into the tail region. This so-called 'intermediate cell mass' is the intra-embryonic red blood cell anlage in many of the species.

The peripheral cells of the mass as claimed by Swaen and Brachet, or the mesenchyme about the mass, Sobotta, form a vascular endothelium which encloses the central early blood corpuscles. In individuals without a circulation the erythroblasts arise in a normal manner in this centrally located position and become erythrocytes or red blood corpuscles filled with haemoglobin. Typical vascular endothelium completely surrounds the erythrocytes which, instead of being swept away as usual by the circulating current, remain in their place of origin. All of the early blood forming cells of this intermediate mass give rise to red corpuscles and never to white blood cells.

6. Contrary to the opinion of most recent observers on blood development in Teleosts, the Fundulus embryos both with and without a circulation, possess blood islands on the posterior and ventral portions of the yolk-sac. These blood islands are formed by wandering mesenchymal cells which migrate out from the caudal region of the embryo. They represent all that remains on the yolk-sac of the peripheral mesoderm in the Teleosts and probably wander away from mesoderm related to that of the intermediate cell mass. The intermediate cell mass may possibly represent the bulk of the peripheral mesoderm which is here included within the embryonic body, while in other meroblastic eggs it is spread out posteriorly over the yolk. The only mesodermal elements of the yolk-sac in Fundulus are the independent wandering mesenchyme cells, some of which group themselves to form the blood islands, while others give rise to the yolk vessel endothelium, and still other wandering cells develop into the chromatophores.

7. The non-circulating red-blood corpuscles within the embryo remain in a fully developed condition for eight or ten days and then undergo degeneration. In an old embryo of sixteen days it is sometimes found that very few of the corpuscles in the intermediate mass are still present and these are degenerate. The vascular endothelium has been lost and numerous mesenchyme cells have wandered in to lie among the corpuscles.

On the yolk-sac the corpuscles no doubt have a better oxygen supply and here they maintain their color longer, but finally also present a degenerate appearance with small densely staining nuclei and cell bodies much reduced in size.

8. Vascular endothelium arises *in loco* in many parts of the embryonic body other than those localities in which blood cells form. The endothelium is in all cases utterly incapable of giving rise to any type of blood cell. This incapacity cannot be attributed to the abnormal condition of the embryo, since true blood cell anlagen in the same specimen produce blood corpuscles in abundance.

The lining of the vessel walls in the fish embryo has no blood forming function.

9. Neither lymphocytes nor other types of white blood corpuscles, have been found to arise in the yolk-sac blood islands, nor within the intermediate cell mass. The embryonic white blood cells are found most abundantly in the anterior body and head regions, and there occupy extra-vascular positions usually lying among the mesenchymal cells.

The sources of origin of the white and red blood corpuscles in Fundulus embryos are distinct, and these two different types of cells cannot be considered to have a monophyletic origin except in so far as both arise from mesenchymal cells.

The adult blood of Fundulus contains lymphocytes and several varieties of granular leucocytes.

10. There is evidence to indicate that definite environmental conditions are necessary for blood cell proliferation or multiplication. Blood cells do not normally divide when completely enclosed by vascular endothelium. This is the key to the shifting series of so-called haematopoetic organs found during embryonic development.

Erythroblasts, embryonic red blood corpuscles, lying about spaces unenclosed by vascular endothelium, proliferate steadily and give off their products into the space from which they find their way into the embryonic vessels. Should such an erythroblast be carried by the circulation to another unlined space, it may become arrested there and again undergo a series of divisions, giving rise to other erythroblasts. When, however, these spaces become lined by endothelium, the blood cell reproduction stops.

In most embryos the earliest blood cell formation occurs in the yolk-sac blood islands. The cells in these islands continue to divide until they become surrounded by endothelium, then the yolk-sac blood islands lose their haematopoetic function and become a vascular net through which the blood circulates. The liver now takes up the rôle of harboring dividing blood cells within its tissue spaces. When these spaces become vascularized by endothelium, here again the blood cells no longer multiply but merely circulate.

Finally, in the mammalian embryo, one organ after another ceases

to offer the necessary harbor for dividing blood cells, until the red bone marrow is the only tissue presenting the proper relationship of spaces and vessels, and here alone the erythropoetic function exists to supply the red blood cells for the entire body circulation. The red blood corpuscles are always produced so as to be delivered into the vessels and thus very soon occupy an intra-vascular position and cease to divide, while the white blood cells arise and remain for some time among the mesenchymal tissue cells in an extra-vascular position.

11. Lymphocytes and leucocytes, so-called white blood cells, along with the invertebrate amoebocytes, are all generalized more or less primitive wandering cells, and are almost universally distributed through out the metazoa.

Erythrocytes, red blood corpuscles, are very highly specialized cells with a peculiar oxygen carrying function, due to their haemoglobin content. In contrast to the universal distribution of the leucocytes, the erythrocytes, the red corpuscles, are only found in the vertebrate phylum and in a few of the higher invertebrate groups. Yet in these invertebrates the oxygen carrying blood cell never presents the typically uniform appearance of the vertebrate erythrocyte. The oxygen carrying function in many invertebrates is confined to the liquid plasma.

The typical vascular endothelial cell is widely distributed in the animal kingdom and appears to be a simple slightly modified mesenchymal cell.

These three very different types of cells all seem to arise from mesoderm—the mesenchyme. Yet the present investigation would indicate that each arises from a distinctly separate mesenchymal anlage. The erythrocyte, red cell, anlage is localized and perfectly consistent in the quality of its production. The lymphocyte and leucocyte, white cell, anlage is more diffusely arranged and not definitely localized in any particular cells group. The vascular endothelium appears to be formed *in loco* in almost all parts of the embryonic body, and its formation is absolutely independent of a circulating fluid or the presence of blood cells.

On the yolk-sac of Fundulus embryos one finds four distinctly different products, red blood corpuscles, endothelium, and two varieties of chromatophores, differentiating from the apparently similar wandering mesenchymal cells. The environment in which the four types differentiate is identical as far as is possible to determine, and the only explanation of their various modes of differentiation is that the original mesenchymal cells that wandered out were already of four potentially different classes. These differences in potentiality within the cells pro-

duced the four different types of structure in one and the same environment. The four types of cells are then in an embryological sense polyphyletic in origin.

Therefore, *vascular endothelium, erythrocytes and leucocytes, although all arise from mesenchyme, are really polyphyletic in origin; that is, each has a different mesenchymal anlage.* To make the meaning absolutely clear, we may consider the origin of the liver and pancreas cells a parallel case, both arise from endoderm but each is formed by a distinctly different endodermal anlage, and if one of these two anlagen is destroyed the other is powerless to replace its product.

A more complete account of this work, with a discussion of the literature bearing on the subject, appears in the September and November numbers of the *American Journal of Anatomy.*

NOTICES OF BIOGRAPHICAL MEMOIRS

The following biographical memoirs have been published by the Academy since the last notices of such memoirs appeared in the June number of the Proceedings.

HENRY MORTON (1836–1902). By EDWARD L. NICHOLS. *Biographical Memoirs of the National Academy*, vol. 8, pp. 143–151.

This Memoir consists in a description of the life-work of Henry Morton. It includes an estimate of his personality by Mr. Thomas A. Janvier quoted from the Morton Memorial Volume published by the Alumni Association of the Stevens Institute of Technology of which Mr. Morton was the first president.

PETER LESLEY (1819–1903). By W. W. DAVIS. *Biographical Memoirs of the National Academy*, vol. 8, pp. 155–240.

This Memoir discusses the life-work of Peter Lesley, under the headings: Ancestry; Boyhood; First Geological Work; Early Appalachian Study; Theology and Geology at Princeton; A Year Abroad; Preaching in Pennsylvania; Geological Work in Boston; Preaching in Milton; Engagement and Marriage; The Lyman Family; Peter and Susan Lesley; Married Life in Milton; Lesley's Hobby, "Arkism;" Return to Pennsylvania and Geology; Anti-Slavery Opinions; Professional Work, its Pleasures and Pains; "Coal and its Topography;" Catastrophic Views; Soil-Creep; Lesley as a Draftsman; Personal Opinions; The American Philosophical Society; The Iron Manufacturers' Guide; A Later Estimate of Rogers; Personal Items; Lowell Institute Lectures; Man's Origin and Destiny; Lesley as a Philologist; "Five Types of Earth Surface;" Two Years Abroad; More Personal Items; A Characteristic Incident; Theoretical Views in Professional Reports; The University of Pennsylvania; Proofreading; The "Second Survey;" The Drudgery of Survey Work; Distractions and Entertainments; The American Association; Trips to Europe; The Dictionary of Fossils; Summary of Pennsylvania Geology; Recognition; Lesley in his Old Age.

At the close the author of the Memoir presents a personal appreciation of Mr. Lesley.